

Medical Applications of Stem Cell Technology in Diseases of Blood and the Circulatory System

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Abstract: The discovery of stem cells heralded great improvements in the prognosis of a number of patients, in particular those having haematological diseases, the classic example being, of course leukaemia. After these encouraging results, scientists are researching ways of manipulating stem cell technology in order to treat more effectively diseases which decimate affluent societies following the epidemic of obesity, such as cardiovascular diseases for example. Furthermore, recent studies give hope in the fight against autoimmune diseases, as well as sickle-cell anaemia, even going some way towards outlining methods of providing a cure, hopefully in the not-too-distant future.

Key words: Stem cells, leukaemia, cardiovascular diseases, autoimmune diseases, sickle-cell anaemia

INTRODUCTION

Stem cells are defined as clonogenic self-renewing progenitor cells that have the ability to divide from an indefinite period and can give rise to one or more differentiated cell types (Vejajiva, 2002). This ability is known as developmental plasticity and as Table 1 shows, differs in the different types of stem cells.

Leukaemia

Non Hodgkin's Lymphoma (NHL): Traditional methods of treating NHL involve transplants of stem cells obtained from bone marrow, after high doses of chemotherapeutic drugs which kill off the patient's bone marrow. However, nowadays, stem cells can also be collected from peripheral blood, which has the advantages of not requiring to anaesthetise the patient in order to recover the cells and the blood counts may potentially recover faster after chemotherapy.

Peripheral Blood Stem Cell Transplants (PBSCT) have been shown to be effective in aggressive cases of lymphoma, which does not respond to treatment or which is likely to come back (although the patient is in remission). Such patients, who effectively have poorer prognoses, can achieve complete remission after marrow-ablative chemotherapy, followed by PBSCT (Kirit *et al.*, 2000).

Comparisons between the pros and cons of PBSCT versus those of bone marrow transplant show that the former has an advantage over bone marrow

transplantation in terms of neutrophil recovery and this can possibly be applied to platelet engraftment (Lewis, 2005).

Stem cell transplants can be divided into:

- Autologous transplants, where stem cells are obtained from the patient and reinjected after marrow-ablative chemotherapy.
- Allogenic transplants, where stem cells are obtained from a donor (may be a sibling, or even a non-related donor), who is found out to be a match. This type of transplant is sometimes done if the patient has a relapse after an autologous transplant, in order to avoid returning the patient's own cancerous cells. However, it is worth noting that allogeneic transplants have more side effects and complications and the number of people suitable for this treatment is restricted.

Research is still being carried out to establish the pros and cons of the 2 protocols.

Acute Myeloblastic Leukaemia (AML): Treatment for AML follows basically the same pattern as for NHL. In this case, the safety and efficacy of myeloablative therapy followed by autologous PBSCT was demonstrated as early as in 1997 by Gondo's team who studied 60 patients suffering from AML (Gondo *et al.*, 1997). High-dose chemotherapy consisting of busulfan (16 mg kg^{-1}), etoposide (40 mg kg^{-1}) and cytosine

Table 1: A comparison of the different types of stem cells in terms of their plasticity

Level of plasticity	Type of cells	Cell types obtainable
Totipotent	Zygote	All cell types-develops into embryo
Pluripotent	a) Embryonic Stem Cells (ES cells)	All cell types-does not develop into an embryo
Pluripotent	b) Umbilical Stem Cells	Same as ES cells
Pluripotent to multipotent	Adult Stem Cells	
	a) Haematopoietic stem cells	Red, white blood cells, immune cells, others?
	b) Mesenchymal stem cells	Lipocytes, cartilage, bone, tendon and ligaments, myocytes, skin cells, neurons
	c) Others (methods of collection still not very well-characterized)	Neurons, hepatocytes, pneumocytes...
	d) Transdifferentiation (occurs under special conditions)	Crossing of germ layers

arabinoside (3 g/m 2×4) (BEA regimen) was used for pretransplant conditioning in 13 patients, whereas for the remaining 47 patients, growth factors (for example Granulocyte Colony-Stimulating Factor (G-CSF) were administered during conditioning.

This study as followed by others, notably de la Rubia's in 1999 which compared the clinical results of two consecutive protocols including Autologous Blood Stem Cell Transplantation (ABSCT) for patients with de novo AML in first complete remission (Rubia *et al.*, 1999). In the first one (group A-20 patients), PBSC were collected after induction and consolidation chemotherapy courses and ABSCT was performed immediately thereafter. In the subsequent 25 patients (Group B), PBSC were collected after consolidation alone and a further chemotherapy course with intermediate dose cytarabine (Ara-C 1 g/m²/12 h×3 days) and mitoxantrone (12 mg/m²/ d×3 days) was administered as early intensification. From the results obtained, it was noted that haematopoietic engraftment was slightly quicker in Group B. Furthermore, autologous transplants with PBSC collected after consolidation chemotherapy were still associated with a high Rate of Relapse (RR). Having said that, the actuarial 4-year Disease-Free Survival (DFS) was not significantly different between the 2 groups (32 v 18%).

AUTOIMMUNE DISEASES

Multiple Sclerosis (MS): Widespread demyelination is a hallmarks of MS. The multifocal nature of this chronic inflammatory disease of the central nervous system complicates cellular therapy and puts emphasis on both the donor cell origin and the route of cell transplantation. In 2003, an Italian research team established syngenic adult neural stem cell cultures and injected them into an animal model of MS (Experimental Autoimmune Encephalomyelitis (EAE) in the mouse) using either of 2 protocols; intravenously or intracerebroventricularly. In both cases, significant numbers of donor cells entered into demyelinating areas of the central nervous system and differentiated into mature brain cells. Within these areas, oligodendrocyte progenitors markedly increased,

with many of them being of donor origin and actively remyelinating axons. Furthermore, a significant decrease of astrogliosis and a marked reduction in the extent of demyelination and axonal loss were observed in transplanted animals, when these animals were studied using MRI, after using iron to magnetically label neural stem cells of adult mice. The functional impairment caused by EAE was almost abolished in transplanted mice, both clinically and neurophysiologically, leading to hopes that stem cell transplants could be the answer towards finding a cure for MS (Pluchino *et al.*, 2003).

Systemic Lupus Erythematosus (SLE): SLE is a chronic inflammatory disorder that can involve any organ system, but most commonly affects joints, skin and kidneys. Research has suggested autologous haematopoietic stem cell transplants preceded by high-dose chemotherapy can arrest progression of severe autoimmune diseases. Between 1995 and 2003, A Gratwohl and his team evaluated the toxicity and disease response in 473 patients with severe autoimmune disease treated with autologous HSCT (Gratwohl *et al.*, 2005) by noting survival, transplant-related mortality, treatment response and disease progression in the said patients. The results were found to support the hypothesis that autologous HSCT can alter disease progression in severe autoimmune disease.

In fact, experimental data and early phase I/II studies where SLE was treated with autologous HSCT gave promising results (Burt *et al.*, 2003). Unfortunately, clinical trials phase II performed in the past, have shown high morbidity and mortality, which was to be expected in a population already at increased risk for thrombosis, vasculitis and atherosclerosis.

Juvenile Chronic Arthritis (JCA): The logic behind using stem cells transplants for JCA is identical to that used for other autoimmune diseases. Since the patient's immune system attacks the patient's body, the logic is that the patient is given a large dose of chemotherapy to kill off defective cells and AHSCT is used to furnish the patient with a newly minted immune system.

For this particular disease, things appear to have advanced quite a lot with a successful clinical trial being reported quite some time ago in 1999, in *The Lancet*. In this experiment, four children with severe forms of JCA treated with AHSCT. Of these four patients, three children had systemic JCA and one child had polyarticular JCA. One month before AHSCT, unprimed bone marrow was taken, after which T-cell depletion of the graft was done with CD2 and CD3 antibodies. A preparative regimen of antithymocyte globulin (20 mg kg⁻¹), cyclophosphamide (200 mg kg⁻¹) and low-dose total body irradiation (4 Gy) were used to destroy the patients' immune system. Methotrexate and cyclosporin were stopped before AHSCT while prednisone was tapered after 2 months. (Wulffraat *et al.*, 1999).

From the results obtained, it was shown that patients showed a drug-free follow-up of 6-18 months with a marked decrease in joint swelling, pain and morning stiffness. Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP) and Hb returned to almost normal values within 6 weeks. In spite of the T-cell depletion, there was a rapid immune reconstitution in three out of four children. AS is the case whenever patients are immunosuppressed and even more so in cases like this where the immune system is destroyed, patients are very vulnerable to infection. In fact, in this experiment, 2 patients developed a limited varicella zoster virus eruption, which was treated by aciclovir (Wulffraat *et al.*, 1999).

This successful research project demonstrated that AHSCT for severe JCA was well tolerated and induced a remission of disease in four children with JCA that was resistant to conventional treatment (Wulffraat *et al.*, 1999).

CARDIOVASCULAR DISEASE

Myocardial Infarctions (MI): In 2004, cardiologist Joshua Hare and his team presented results from an animal study conducted at Johns Hopkins, which showed that stem cell therapy can be used effectively to treat MIs in pigs. Stem cells were taken from another pig's bone marrow and injected into the animal's damaged heart and it was found that they were able to restore the heart's function to its original condition (Hare *et al.*, 2005).

In a controlled study of 14 pigs, seven received therapy and another seven received only a placebo. The researchers found that injections of BMSC directly into heart muscle, recently damaged by an MI, produced a nearly full recovery after 2 months.

Recovery was measured for the seven treated animals as full restoration of heart muscle contraction to levels

existing prior to infarction. Indeed, dead scar tissue was reported to have nearly disappeared after therapy and only a small trace of the MI was left.

In contrast, for the seven animals in the control group that did not receive therapy, no recovery was observed and the animals' condition worsened, leading to the development of congestive heart failure within two months after the MI (Hare *et al.*, 2005).

While on the one hand this news appears to be short of miraculous, recently 3 separate studies appeared in the New England Journal of Medicine, which appeared to discredit this evidence, reporting that there was little evidence to justify the administration of adult stem cells to MI patients.

In a study led by Dr. Lunde, patients with acute MI of the anterior wall treated with percutaneous coronary intervention, were randomly assigned to the group that underwent intracoronary injection of autologous BMSC or to the control group, in which neither aspiration nor sham injection was performed. Electrocardiogram-gated Single-Photon-Emission Computed Tomography (SPECT) and echocardiography at baseline and Magnetic Resonance Imaging (MRI) were used 2-3 weeks after the infarction to assess left ventricular function. These procedures were repeated 6 months after the MI.

Results obtained in this study showed that BMSCT after MIs did not significantly effect the patients in the study, given that 2 groups did not differ significantly in changes in left ventricular end-diastolic volume or infarct size and had similar rates of adverse events (Lunde *et al.*, 2006).

This result was echoed by 2 separately conducted studies, published in the same journal, namely one led by Schächinger *et al.* (2006) and another led by Assmus *et al.* (2006).

All 3 studies made use of protocols which were scientifically correct, thus undermining support for stopping ES cell research, given that the results obtained by adult stem cells are highly questionable, when seen in this light.

Stroke: Treatment for stroke aims to restore function in the diseased human brain by replacing dead neurons with new neurons through transplantation or stimulation of neurogenesis from endogenous stem/precursor cells. To repair the human brain after stroke may seem impossible because of the atrophy and loss of many different neuron and glial cell types. However, it can be argued that reestablishment of even only a fraction of damaged neuronal circuitries might have significant implications.

Current research gives quite encouraging results in that the newly generated neurons were found to be able

to migrate toward the damage and adopt the phenotype of those cells that have died, at least to some extent, as demonstrated in 2 different studies (Modo *et al.*, 2002; Arvidsson *et al.*, 2002).

Having said that, it is important to note that many basic issues remain to be solved, such as for example the mechanism on endogenous repair which occurs after stroke, how to control stem cell proliferation and differentiation into specific phenotypes, induce their integration into existing neural and synaptic circuits and to optimize the functional recovery in animal models of stroke (Lindvall and Kokaia, 2004).

SICKLE CELL ANAEMIA

In this field, research is moving at a fast pace in quite a number of different therapy. In fact, haemopoietic blood transfusions (using stem cells obtained from the bone marrow) were the first line of research. Such transfusions require siblings who are a good genetic match. This led to researchers looking into stem cell transplants using US cells from unrelated donors. Vichinsky (2002) in fact, managed to perform this with apparent success meaning that this approach has turned out to be quite promising.

Statistics in fact, show that about 150 children worldwide have had blood stem cell transplants and about 85% of them appear to be cured of the disease. It is important to note however, that this approach carries a high risk: About 5% of children who underwent bone marrow transplants died while the transplant failed in another 10% (data obtained from the National Institutes of Health. Hematopoietic Cell Transplantation, in Management of Sickle Cell Disease. NIH Publication Number 02-2117, revised May 28, 2002, accessed 4/19/04).

Finally, it is worth noting a novel approach undertaken by team of scientists at Memorial Sloan-Kettering Cancer Centre, who have combined gene therapy with stem cell research and RNA interference in an attempt to genetically reverse sickle cell disease in human cells. Adult stem cells were obtained from sickle cell patients and were cultured in the lab. A viral vector was constructed to that it carried a therapeutic globin gene, harbouring a small interfering RNA precursor specific for beta S-globin and designed to suppress abnormal Haemoglobin (Hb) formation. The vector was introduced in the cell culture, resulting in suppression of the production of HbS and leading to the production of normal Hb in RBCs (Samakoglu *et al.*, 2006).

CONCLUSION

While acknowledging that great advances have been made, especially with regard to blood and the circulatory system, it is important to realize that there are still great obstacles to overcome before some of the therapies highlighted out by experimental evidence available today, become a reality. Furthermore, stem cell therapies need to be made commercially viable before they can be used to treat the general public.

Having said that, stem cells therapies offer hope to patients of diseases for which there is currently no cure, in manner of more effective treatment or even, for some diseases, offering a cure; hence resulting inevitably in a better quality of life for the both patient and the people surrounding him/her.

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